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CLAIMS

1. Process for specific replacement of a gene, in particular by targetting of a DNA, called insertion DNA constituted by a part of a gene capable of being made functional or whose functioning may be made more effective, when it is recombined with a complementing DNA so as thus to provide a complete recombinant gene in the genome of a eucaryotic cell characterized in that

- the insertion site is situated in a selected gene, called selected recipient gene, containing the complementing DNA, and in that
- eucaryotic cells are transfected with a vector containing an insert itself comprising the insertion DNA and two so-called "flanking" sequences on either side of the insertion DNA homologous, respectively, to two genomic sequences which are adjacent to the desired insertion site in the recipient gene,
- the insertion DNA being heterologous with respect to the recipient gene, and
- the flanking sequences, being selected from those which constitute the above-mentioned complementing DNA and which allow, by means of homologous recombination with corresponding sequences in the recipient gene, the reconstitution of a complete recombinant gene in the genome of the eucaryotic cell.

2, Process according to Claim 1, the said insertion DNA containing either a coding sequence or a regulatory sequence, characterized in that

- the insertion site is located in a selected gene called recipient gene and in that
- eucaryotic cells are transfected with a vector containing an insert itself comprising the insertion DNA and two so-called "flanking" sequences on either side of the insertion DNA homologous, respectively, to two genomic sequences which are adjacent to the desired insertion site in the recipient gene,
- the insertion DNA being heterologous with respect to the recipient gene and,
- the flanking sequences being selected so as to make possible by

homologous recombination depending on the circumstances, of either the expression of the coding sequence of the entire insertion DNA under the control of the regulatory sequences of the recipient gene, or the expression of a coding sequence of the recipient gene under the control of the regulatory sequences of the insertion DNA.

3. Process according to Claim 1 or 2, characterized in that the insertion DNA contains a coding sequence lacking a regulatory element , in particular a promoter which is intrinsic to it.

4. Process according to any one of the Claims 1 to 3, characterized in that the recipient gene is present in the genome of the eucaryotic cell in at least two copies.

5. Process according to any one of the Claims 1 to 4, characterized in that each of the flanking sequences has a length greater than 150 base pairs and shorter than the length of the recipient gene.

6. Process according to any one of the Claims 1 to 5, characterized in that the eucaryotic cells are embryonic stem (E.S.) cells.

7. Process according to one of the preceding Claims, characterized in that the gene to be inserted is a gene heterologous with respect to the species transfected.

8. Process according to one of the preceding Claims, characterized in that the vector contains sequences intercalated between the gene to be inserted and the flanking sequences.

9. Process according to Claim 8, characterized in that the intercalating sequences contain a sequence coding for a selective agent making possible the selection of the transformants and, where appropriate, a marker gene, for example the Lac Z.

10. Process according to one of the preceding Claims, characterized in that the transfection is carried out by electroporation.

11. Process according to one of the preceding Claims, characterized in that the technique of Polymerase Chain Reaction (P.C.R.) is used to amplify the DNA sequence of the locus at which the insertion is made in order to verify that the insertion occurred

at the desired site.

12. Process according to Claim 1, characterized in that the insertion DNA contains, between the flanking sequences, a DNA sequence designed to be recombined with the complementing DNA in the recipient gene in order to provide a recombinant gene, on the one hand, and, on the other hand, a sequence coding for a selective agent making possible the selection of the transformants and a promoter allowing the expression of the selective agent, the recipient gene and the recombinant gene coding for products of expression not conferring a selectable phenotype.

13. Process for the production of transgenic animals, characterized in that E.S. cells are transfected by the procedure according to one of the Claims 1 to 12 and selected for the homologous recombination event, namely the correct integration of the foreign gene, the cells are injected into embryos at a stage at which they are capable of integrating the transfected cells, for example the blastocyst stage, the latter are then reimplanted in a surrogate mother and the chimeric individuals obtained at the term of pregnancy and in which the colonization of the germ line by the E.S. cells is observed, are mated in order to obtain transgenic animals heterozygous for the replaced gene.

14. Plasmid capable of effecting the targetted insertion of a gene, called insertion gene , in the genome of a eucaryotic cell, characterized in that it contains an insert itself comprising the insertion gene and two so-called "flanking" sequences on either side of the gene to be inserted homologous, respectively, with two genomic sequences which are adjacent to the desired insertion site in the recipient gene.

15. Plasmid according to Claim 14, characterized in that the insert contains, between the flanking sequences, a DNA sequence designed to be recombined with the complementing DNA in the recipient gene, on the one hand, and, on the other hand, a sequence coding for a selective agent making possible the selection of the transformants and a promoter allowing the expression of the selective agent, the DNA sequence designed to be recombined with the complementing DNA being

other than a gene coding for a selective agent.

16. Plasmid pGN as illustrated in figure 1.

17. Eucaryotic cells transformed by the procedure of Claim 1.

18. Cells according to Claim 17, characterized in that they are E.S. cells.

19. Transgenic animal in which a single copy of a gene which is present in the genome in at least two copies was inactivated by the insertion of a gene which is different from the inactivated gene, the inserted gene being inserted in a position which allows the expression of this gene under the control of regulatory sequences of the inactivated endogenous gene.

20. Use of the procedure according to any one of the Claims 1 to 12 for gene therapy.

21. Use of the procedure according to any one of the Claims 1 to 12 for the production of transgenic animals.

22. Use of the procedure of Claim 9 for genetic marking of animals.

23. Use of the procedure of Claim 13 for the screening of pharmaceutical products presumed to have an activity with respect to the products of expression of a pathological gene associated with a disease, characterized in that the gene to be inserted is constituted by the pathological gene or a fragment of the latter and in that the pharmaceutical product to be tested is administered to the transgenic animal for the purpose of evaluating its activity on the disease.